

**#9210** Store at -20°C

# PhosphoPlus® p38 MAP Kinase (Thr180/Tyr182) Antibody Kit

✓ 20 western blots



**Orders** ■ 877-616-CELL (2355)  
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

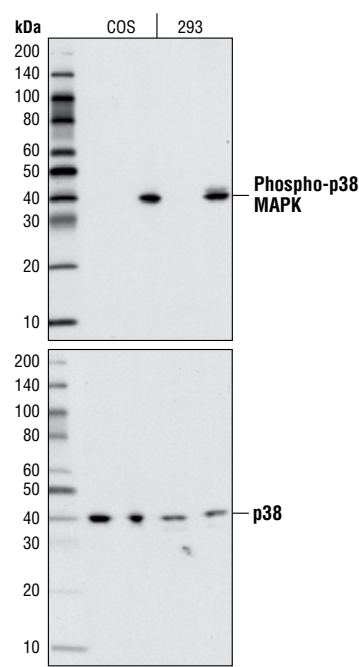
Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb	4511	200 µl	43 kDa	Rabbit
p38 MAP Kinase Antibody	9212	200 µl	43 kDa	Rabbit
p38 MAP Kinase Control Cell Extracts	9213	60 µl		
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked Antibody	7075	250 µl		Goat
Biotinylated Protein Ladder Detection Pack	7727	250 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	10 ml		

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Background:** p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase which participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAP kinase, p38 $\alpha$ ,  $\beta$ ,  $\gamma$  (also known as ERK6 or SAPK3) and  $\delta$  (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors (1-5). MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6) and MEF2 (5-8).

**Specificity/Sensitivity:** Phospho-p38 MAP Kinase Antibody detects phosphorylated threonine 180 and tyrosine 182 of p38 MAP Kinase but does not appreciably cross-react with the corresponding phosphorylated forms of either p42/44 MAP Kinase or SAPK/JNK.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr180/Tyr182 of human p38 MAPK. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human p38 MAPK (p38 MAP Kinase Antibody).

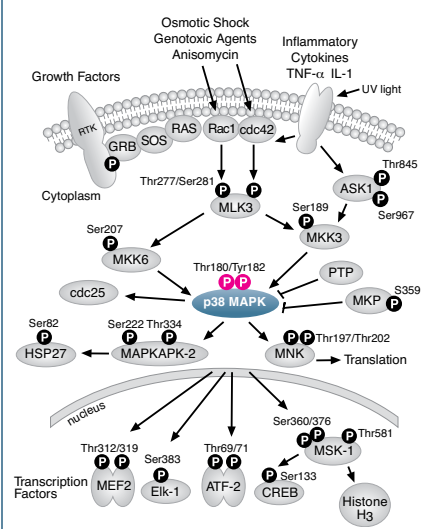


Western blot analysis of extracts from COS and 293 cells, untreated or UV-treated, using **Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb #4511** (upper) or **p38 MAPK Antibody #9212** (lower).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. *Do not aliquot the antibody.*

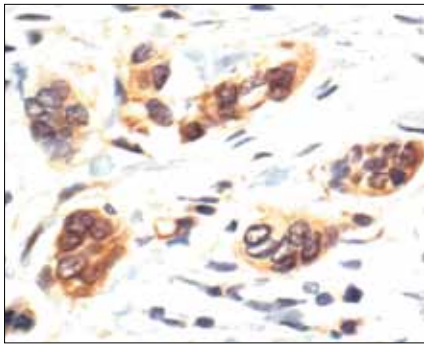
**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

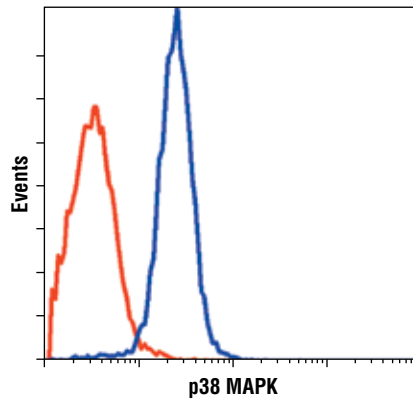


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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear and cytoplasmic localization, using **p38 MAP Kinase Antibody #9212**.



Flow cytometric analysis of Jurkat cells, using **p38 MAP Kinase Antibody #9212** (blue) compared to a nonspecific negative control antibody (red).

#### Selected Application References:

- De, A.K. et al. (2000) Exaggerated human monocyte IL-10 concomitant to minimal TNF- $\alpha$  induction by heat-shock protein 27 (Hsp27) suggests Hsp27 is primarily an antiinflammatory stimulus [In Process Citation]. *Journal of Immunology* 165, 3951–3958. Application: W.
- Legos, J.J. et al. (2001) SB 239063, a novel p38 inhibitor, attenuates early neuronal injury following ischemia. *Brain Research* 892, 70–77. Application: W.
- Liu, F. et al. (2002) GnRH activates ERK1/2 leading to the induction of c-fos and LH $\beta$  protein expression in L $\beta$ T2 cells. GnRH activates ERK1/2 leading to the induction of c-fos and LH $\beta$  protein expression in L $\beta$ T2 cells. *Mol. Endocrinol.* 16, 419–434. Application: W.
- Lee, Y.W. et al. (2000) 2-Amino-3-methylimidazo[4,5-f]quinoline inhibits nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 cells by blocking p38 kinase activation. *Cancer Lett.* 156, 133–139. Application: W.
- Griego, S.D. et al. (2000) Role of p38 mitogen-activated protein kinase in rhinovirus-induced cytokine production by bronchial epithelial cells [In Process Citation]. *Journal of Immunology* 165, 5211–5220. Application: W.
- Novitskaya, V. et al. (2000) Oligomeric forms of the metastasis related Mts1(S100A4) protein, stimulate neuronal differentiation in cultures of rat hippocampal neurons. *Journal of Biological Chemistry.* Application: W.
- Watanabe, H. et al. (2001) Transcriptional Cross-talk between Smad, ERK1/2, and p38 Mitogen-activated Protein Kinase Pathways Regulates Transforming Growth Factor- $\beta$ -induced Aggrecan Gene Expression in Chondrogenic ATDC5 Cells. *Journal of Biological Chemistry* 276, 14466–14473. Application: W.

#### Background References:

- (1) Rouse, J. et al. (1994) *Cell* 78, 1027–1037.
- (2) Han, J. et al. (1994) *Science* 265, 808–811.
- (3) Lee, J.C. et al. (1994) *Nature* 372, 739–746.
- (4) Freshney, N.W. et al. (1994) *Cell* 78, 1039–1049.
- (5) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420–7426.
- (6) Zervos, A.S. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 10531–10534.
- (7) Zhao, M. et al. (1999) *Mol. Cell. Biol.* 19, 21–30.
- (8) Yang, S.H. et al. (1999) *Mol. Cell. Biol.* 19, 4028–4038.

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.